No.



200600103

<u>THE UNITED STATES OF AMERICA</u>

TO ALL TO WHOM THESE PRESENTS SHAIL COME:

Atah State Unibersity

MILEONS, THERE HAS BEEN PRESENTED TO THE

Secretary of Agriculture

AN APPLICATION REQUESTING A CERTIFICATE OF PROTECTION FOR AN ALLEGED DISTINCT VARIETY OF SEXUALLY REPRODUCED, OR TUBER PROPAGATED PLANT, THE NAME AND DESCRIPTION OF WHICH ARE CONTAINED IN THE APPLICATION AND EXHIBITS, A COPY OF WHICH IS HEREUNTO ANNEXED AND MADE A PART HEREOF, AND THE VARIOUS REQUIREMENTS OF LAW IN SUCH CASES MADE AND PROVIDED HAVE BEEN COMPLIED WITH, AND THE TITLE THERETO IS, FROM THE REGORDS OF THE PLANT VARIETY PROTECTION OFFICE, IN THE APPLICANT(S) INDICATED IN THE SAID GOPY, AND WHEREAS, UPON DUE EXAMINATION MADE, THE SAID APPLICANT(S) IS (ARE) ADJUDGED TO BE ENTITLED TO A CERTIFICATE OF PLANT VARIETY PROTECTION UNDER THE LAW.

NOW, THEREFORE, THIS CERTIFICATE OF PLANT VARIETY PROTECTION IS TO GRANT UNTO THE SAID APPLICANT(S) AND THE SUCCESSORS, HEIRS OR ASSIGNS OF THE SAID APPLICANT(S) FOR THE TERM OF TWENTY YEARS FROM THE DATE OF THIS GRANT, SUBJECT TO THE PAYMENT OF THE REQUIRED FEES AND PERIODIC REPLEMISHMENT OF VIABLE BASIC SEED OF THE VARIETY IN A PUBLIC REPOSITORY AS PROVIDED BY LAW, THE IGHT TO EXCLUDE OTHERS FROM SELLING THE VARIETY; OR OFFERING IT FOR SALE, OR REPRODUCING IT, OR PORTING IT, OR EXPORTING IT, OR CONDITIONING IT FOR PROPAGATION, OR STOCKING IT FOR ANY OF THE VETURE FOR THE PLANT VARIETY PROTECTION ACT. IN THE UNITED STATES SEED OF THIS VARIETY BESOLD BY VARIETY NAME ONLY AS A CLASS OF CERTIFIED SEED AND (2) SHALL CONFORM TO THE GENERATIONS SPECIFIED BY THE OWNER OF THE RIGHTS. (84 STAT. 1542, AS AMENDED, 7 U.S.C. 2321

BARLEY

'Goldeneye'

In Testimone Thereof, I have hereunto set my hand and caused the seal of the Hint Bariety Frotestion Office to be affixed at the City of Washington, D.C. this sixth day of December, in the year two thousand and six.

Attost:

Commissioner

Commissioner
Plant Variety Protection Office
Spricultural Warbeting Service

erotary of Agriculture

15. GENUS AND SPECIES NAME OF CROP Hordeum vulgare	17. IS THE VARIETY A FIRST GENERATION HYBRID?	IF SO, PLEASE GIVE THE ASSIGNED USDA-APHIS REFERENCE NUMBER FOR TH APPROVED PETITION TO DEREGULATE THE GENETICALLY MODIFIED PLANT F COMMERICALIZATION.
 CHECK APPROPRIATE BOX FOR EACH ATTA (Follow instructions on reverse) 	ACHMENT SUBMITTED	20. DOES THE OWNER SPECIFY THAT SEED OF THIS VARIETY BE SOLD AS A CLASS OF CERTIFIED SEED? (See Section 83(a) of the Plant Variety Protection Act)
a. 🔽 Exhibit A. Origin and Breeding History	of the Variety	YES (If "yes", answer items 21 and 22 below) NO (if "no", go to item 23,
b. Exhibit B. Statement of Distinctness		21. DOES THE OWNER SPECIFY THAT SEED OF THIS VARIETY BE LIMITED AS TO NUMBER OF CLASSES?
c. 📝 Exhibit C. Objective Description of Var	iety	YES NO
d. 📝 Exhibit D. Additional Description of the	Variety (Optional)	IF YES, WHICH CLASSES? ☑ FOUNDATION ☑ REGISTERED ☒ CERTIFIE
e. 📝 Exhibit E. Statement of the Basis of the	e Owner's Ownership	22. DOES THE OWNER SPECIFY THAT SEED OF THIS VARIETY BE LIMITED AS TO NUMBER OF GENERATIONS?
	ed seeds or, for tuber propagated varieties, eposited and maintained in an approved public	YES NO IF YES, SPECIFY THE NUMBER 1,2,3, etc. FOR EACH CLASS.
g. Filing and Examination Fee (\$3,652), in States" (Mail to the Plant Variety Protect	nade payable to "Treasurer of the United ction Office)	FOUNDATION REGISTERED CERTIFIED (If additional explanation is necessary, please use the space indicated on the reverse.)
23. HAS THE VARIETY (INCLUDING ANY HARVES FROM THIS VARIETY BEEN SOLD, DISPOSED OTHER COUNTRIES?	STED MATERIAL) OR A HYBRID PRODUCED D OF, TRANSFERRED, OR USED IN THE U.S. OR	24. IS THE VARIETY OR ANY COMPONENT OF THE VARIETY PROTECTED BY INTELLECTUAL PROPERTY RIGHT (PLANT BREEDER'S RIGHT OR PATENT)?
YES 🗹 NO		YES NO
	FIRST SALE, DISPOSITION, TRANSFER, OR USE NICES. (Please use space indicated on reverse.)	IF YES, PLEASE GIVE COUNTRY, DATE OF FILING OR ISSUANCE AND ASSIGNED REFERENCE NUMBER. (Please use space indicated on reverse.)

The owners declare that a viable sample of basic seed of the variety has been furnished with application and will be replenished upon request in accordance with such regulations as may be applicable, or for a tuber propagated variety a tissue culture will be deposited in a public repository and maintained for the duration of the certificate.

The undersigned owner(s) is(are) the owner of this sexually reproduced or tuber propagated plant variety, and believe(s) that the variety is new, distinct, uniform, and stable as required in Section 42, and is entitled to protection under the provisions of Section 42 of the Plant Variety Protection Act.

representation herein can jeopardize protection and result in penalties SIGNATURE OF **OWNE** NAME (Please print or 1)

(See reverse for instructions and information collection burden statement)

GENERAL: To be effectively filed with the Plant Variety Protection Office (PVPO), ALL of the following items must be received in the PVPO: (1) Completed application form signed by the owner; (2) completed exhibits A, B, C, E; (3) for a seed reproduced variety at least 2,500 viable untreated seeds, for a hybrid variety at least 2,500 untreated seeds of each line necessary to reproduce the variety, or for tuber reproduced varieties verification that a viable (in the sense that it will reproduce an entire plant) tissue culture will be deposited and maintained in an approved public repository; (4) check drawn on a U.S. bank for \$3,652 (\$432 filing fee and \$3,220 examination fee), payable to "Treasurer of the United States" (See Section 97.6 of the Regulations and Rules of Practice.) Partial applications will be held in the PVPO for not more than 90 days, then returned to the applicant as unfilled. Mail application and other requirements to Plant Variety Protection Office, AMS, USDA, Room 401, NAL Building, 10301 Baltimore Avenue, Beltsville, MD 20705-2351. Retain one copy for your files. All items on the face of the application are self explanatory unless noted below. Corrections on the application form and exhibits must be initialed and dated. DO NOT use masking materials to make corrections. If a certificate is allowed, you will be requested to send a check payable to "Treasurer of the United States" in the amount of \$432 for issuance of the certificates. Certificates will be issued to owner, not licensee or agent.

Plant Variety Protection Office Telephone: (301) 504-5518 FAX: (301) 504-5291

Homepage: http://www.ams.usda.gov/science/pvpo/pvpindex.htm

To avoid conflict with other variety names in use, the applicant must check the appropriate recognized authority and provide evidence that name has been cleared by the appropriate recognized authority before the Certificate of Protection is issued. For example, for agricultural and vegetable crops, contact: Seed Branch, AMS, USDA, 10301 Baltimore Avenue, Suite 401 NAL Building, Beltsville, MD 20705. Telephone: (301) 504-5682 http://www.ams.usda.gov/lsg/seed.htm.

ITEM

- 19a. Give:
- (1) the genealogy, including public and commercial varieties, lines, or clones used, and the breeding method;
- (2) the details of subsequent stages of selection and multiplication;
- (3) evidence of uniformity and stability; and
- (4) the type and frequency of variants during reproduction and multiplication and state how these variants may be identified
- 19b. Give a summary of the variety's distinctness. Clearly state how this application variety may be distinguished from all other varieties in the same crop. If the new variety is most similar to one variety or a group of related varieties:
 - (1) identify these varieties and state all differences objectively;
 - (2) attach statistical data for characters expressed numerically and demonstrate that these are clear differences; and
 - (3) submit, if helpful, seed and plant specimens or photographs (prints) of seed and plant comparisons which clearly indicate distinctness.
- 19c. Exhibit C forms are available from the PVPO Office for most crops; specify crop kind. Fill in Exhibit C (Objective Description of Variety) form as completely as possible to describe your variety.
- 19d. Optional additional characteristics and/or photographs. Describe any additional characteristics that cannot be accurately conveyed in Exhibit C. Use comparative varieties as is necessary to reveal more accurately the characteristics that are difficult to describe, such as plant habit, plant color, disease resistance, etc.
- 19e. Section 52(5) of the Act requires applicants to furnish a statement of the basis of the applicant's ownership. An Exhibit E form is available from the PVPO.
- 20. If "Yes" is specified (seed of this variety be sold by variety name only, as a class of certified seed), the applicant MAY NOT reverse this affirmative decision after the variety has been sold and so labeled, the decision published, or the certificate issued. However, if "No" has been specified, the applicant may change the choice. (See Regulations and Rules of Practice, Section 97.103).
- 23. See Sections 41, 42, and 43 of the Act and Section 97.5 of the regulations for eligibility requirements.
- 24. See Section 55 of the Act for instructions on claiming the benefit of an earlier filing date.
- 22. CONTINUED FROM FRONT (Please provide a statement as to the limitation and sequence of generations that may be certified.)
- 23. CONTINUED FROM FRONT (Please provide the date of first sale, disposition, transfer, or use for each country and the circumstances, if the variety (including any harvested material) or a hybrid produced from this variety has been sold, disposed of, transferred, or used in the U.S. or other countries.)
- 24. CONTINUED FROM FRONT (Please give the country, date of filing or issuance, and assigned reference number, if the variety or any component of the variety is protected by intellectual property right (Plant Breeder's Right or Patent).)

NOTES: It is the responsibility of the applicant/owner to keep the PVPO informed of any changes of address or change of ownership or assignment or owner's representative during the life of the application/certificate. The fees for filing a change of address; owner's representative; ownership or assignment; or any modification of owner's name is specified in Section 97.175 of the regulations. (See Section 101 of the Act, and Sections 97.130, 97.131, 97.175(h) of the Regulations and Rules of Practice.)

According to the Paperwork Reduction Act of 1995, an agency may not conduct or sponsor, and a person is not required to respond to a collection of information unless it displays a valid CMB control number for this information collection is 0581-0055. The time required to complete this information collection is estimated to average 1.4 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

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To file a complaint of discrimination, write USDA, Director, Office of Civil Rights, Room 326-W, Whitten Building, 14th and Independence Avenue, SW, Washington, DC 20250-9410 or call 202-720-5964 (voice and TDD). USDA is an equal opportunity provider and employer.

Exhibit A - Origin and Breeding History

Goldeneye

Summer, 1990:

Final cross made at Logan, Utah, by Dr. Rulon S. Albrechtsen Cross number was UT95B 1216

ID633019/'Woodvale'//'Steptoe'//OR3

ID633019=CI9196/CI10119//'Traill'

The order of crosses is as follows,

ID633019 by Woodvale in 1970,

Progeny line UT75B65-504 -generated by the above cross- crossed with Steptoe in 1979,

Progeny line UT84B- B427-2507 -generated by the above cross-crossed with OR3 in 1990. Cross number was UT95B 1216

Winter, 1990-91:

F₁ plants grown in the greenhouse at Logan, Utah.

There was no segregation observed in F_1 plants.

Summers, 1991, Through 1993:

F₂ through F₄ generation plants grown in field conditions at Logan, Utah in space-planted (plants 6 inches apart with 12-inch row spacing) modified bulked populations which were selected for plants possessing the following characteristics:

- · Four or more fertile tillers per plant in space-planted stands
- · Early to mid-season heading date
- · Early to mid-season maturity date
- · Less than 90 cm tall
- · Zero to near-zero lodging
- · Upright stems
- · Desirable plant conformation
- · Plump seeds
- · White aleurone
- · Complete exertion of spike from flag leaf at maturity
- · Tough (not brittle) stem and neck
- · Lemma awns longer than spike
- · Free of barley loose smut (caused by *Ustilago nuda* (Jens.) Rostr.)

· Free of barley covered smut (caused by *Ustilago hordei* (pers.) Lagrh.)

· Moderately free of powdery mildew (caused by *Erysiphe graminis* DC. F sp. Herdei Em. Marchal)

· Goldeneye is susceptible to barley stripe rust (caused by *Puccinia striiformis* Westend)

Selected seed was bulked for each succeeding generation.

Summer, 1994:

 F_5 Plants grown at Logan, Utah in a space planted (plants 6 inches apart with 12 inch row spacing) modified bulked populations and single heads were selected from 157 plants possessing the same characteristics as those listed for the F_2 through F_4 generations.

Summer, 1995:

Seed from the 157 individual selected heads were grown in F6 head rows at Logan, Utah, where all rows were evaluated for the same characteristics as those listed for F_2 through F_5 generations. Only desirable rows were harvested. Seed from harvested rows were subjected to protein evaluation and kernel rating in the laboratory. Row 4087 (identified as UT95B 1216-4087) was selected as a single head row for additional testing. It was found to breed true for rough lemma awns.

Summer, 1996 and 1997:

UT95B 1216-4087 was evaluated for yield and test weight, in addition to the characters listed for the F₆ head rows, in a single-replicate preliminary irrigated yield test (which included Steptoe check plots) grown at Logan, Utah.

Summers, 1998 through 2005:

UT95B 1216-4087 was evaluated in replicated irrigated yield tests at four major irrigated barley production sites in Utah.

Summers, 2001

Through 2003:

UT95B 1216-4087 was evaluated for the same characteristics listed for the preliminary irrigated yield test, in the Western Regional Irrigated Spring Barley Nursery.

Summer 2002:

Selected 100 heads of UT95B 1216-4087 were selected among the F₅₋₁₁ progenies at Logan, Utah to be used for the production of Breeder seed.

Winter, 2002

and 2003: Breeder seed of UT97B 1216-4087 was produced at Yuma,
Arizona, from the selected 100 heads. The 100 single head rows

were rogued for off types, retained rows were harvested in bulk to constitute the breeder seed.

Summer 2003:

Foundation seed of Goldeneye (UT97B 1480-1632) was produced at Cache Junction, Utah from Breeder seed produced in winter 2003. The foundation filed was rogued heavily for questionable plants.

Summer 2004:

Registered seed of Goldeneye was produced.

Summer 2005:

Certified Seed of Goldeneye was produced to be marketed for commercial production in 2006

Goldeneye has been observed to be stable for 8 generations (beginning with the F_6 head row from which it originated in 1998, through the F_{11} foundation seed produced in 2003). There have been no variants observed. Any questionable plants rogued from Breeder and Foundation plantings showed very minor, if any, variation and were likely due to environmental variations. They were removed strictly as a precautionary measure.

Exhibit B - Novelty Statement for Goldeneye

To our knowledge, Goldeneye mostly nearly resembles Millennium and Steptoe barleys. Differences between Goldeneye and the other two varieties include, but are not restricted to, the following characteristics:

- 1. Head shape of Goldeneye is slightly tapered like steptoe, while Millennium is a tapered head shape.
- 2. Head density of Goldeneye [erect (not dense), (2.7-2.9 mm/internode)] is similar to that of Millennium [erect (not dense), (2.4-2.7 mm/internode)], and more dense than Steptoe [lax, (3.2-3.5 mm/internode)].
- 3. Goldeneye has limited overlapping of upper lateral spikelets, while Millennium has some overlap of lateral kernels at the tip of the head which is similar to Steptoe.
- 4. Goldeneye has a rachis edge covered with fewer hairs than Millenium and Steptoe.
- 5. Goldeneye, Millennium, and Steptoe all have glume awns longer than the glumes.
- 6. Goldeneye has no hairs or only a few visible on the ventral surface of the glumes, while Millennium has more visible hairs on the ventral surface of the glumes than Goldeneye, and Steptoe is covered with hairs on the ventral surface of the glumes.
- 7. The lemma base of Goldeneye and Millennium has a depression, while Steptoe has a transverse crease.

On exhibit D, we present additional genotyping of Goldeneye with 32 barley microsatellites or SSR markers. We found polymorphism between Goldeneye vs Aquila or Millenium or Steptoe for eight SSR markers covering four out of the seven chromosomes of barley.

*According to the Paperwork Reduction Act of 1995, an agency may not conduct or sponsor, and a person is not required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0581-0055. The time required to complete this information collection is estimated to average 1.5 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

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U.S. DEPARTMENT OF AGRICULTURE AGRICULTURAL MARKETING SERVICE SCIENCE AND TECHNOLOGY PLANT VARIETY PROTECTION OFFICE BELTSVILLE, MD 20705

OBJECTIVE DESCRIPTION OF VARIETY Barley (Hordeum vulgare L.)

	Dairoy (rioradam raigard Er)	The state of the s
NAME OF APPLICANT (S)	TEMPORARY OR EXPERIMENTAL DESIGNATION	VARIETY NAME
Utah Agricultural Experiment Station	UT95B1216-4087	Goldeneye
ADDRESS (Street and No. or RD No., City, State, Zip Code, and Country	Ku	FOR OFFICIAL USE ONLY
Utah State University 4820 Old Main Hill		PVPO NUMBER
Logan, UT 84322		200600103
PLEASE READ ALL INSTRUCTIONS CAREFUL	LY:	
Place the appropriate number that describes the va when the number is either 99 or less or 9 or less.	rietal character of this variety in the boxes below. Place a	zero in the first box (i.e. 0 9 9 or 0 9)
GROWTH HABIT: 1 = Spring 2 = Facultative Winter 3	3 = Winter Early Growth: 3 1 = Prosti	rate 2 = Semi-Prostrate 3 = Erect
<u></u>	/lid-Season (Betzes) 3 = Late (Frontier) nesse *	
•	nnium *	the second secon
2 No. of Days Later Than	toe *	
3. PLANT: (From Soil Level to Top of Head) 3	ornia Mariout) 3 = Medium Tall (Betzes) * * * *	4 = Tall (Conquest)
No. of Nodes (Originating from Node Collar Shape: 1 = Closed	2 = Present	ed or Open

^{*} A commercial variety grown in the same trial.

200600103 ₹6. LEAF: 1 Basal Leaf Sheath (Seedling): 1 = Glabrous 2 = Pubescent 2 Position of Flag Leaf (At Boot Stage): 2 = Upright 1 = Drooping 3 Waxiness: 1 = Absent (Glossy) 2 = Slightly Waxy 3 = Waxymm Width (First Leaf Below Flag Leaf) cm Length (First Leaf Below Flag Leaf) Anthocyanin in Leaf Sheath: 1 = Absent 2 = Present 6. HEAD: 2 2 = Six-Rowed Type: 1 = Two-Rowed 2 = Erect (Not Dense) 3 = Erect (Dense) Density: 1 = Lax 4 = Other (Specity) ___ 4 = Other (Specify) Shape: 1 = Tapering 2 Strap 3 = Clavate Waxiness 1 = Absent (Glossy) 2 = Slightly Waxy 3 = Waxy3 = 1/4 - 1/2 of Head Lateral Kernels Overlap: 1 = None2 = At Tip Rachis (Halr on Edge): 1 = Lacking 2 = Few 3 = Covered 7. GLUME: 3 1 = 1/3 of Lemma 2 = 1/2 of Lemma 3 = More than 1/2 of Lemma Length: 2 2 = Short 3 = Long Hairs: 1 = None Hair Covering: 1 = None 2 = Restricted to Middle 3 = Confined to Band 4 = Completely Covered 3 = More than Equal to Length of Glumes 1 = Less than Equal to Length of Glumes 2 = Equal to Length of Glumes Awn Surface: 1 = Smooth 2 = Semi-Smooth 3 = Rough 8. LEMMA: 5 Awn: 1 = Awnless 2 = Awnlets on Central Rows, Awnless on Lateral Rows 3 = Short on Central Rows, Awnlets on Lateral Rows 4 = Short (Less than Equal to Length of Spike) 5 = Long (Longer than Spike) 6 = Hooded Awn Surface: 1 = Awnless 2 = Smooth 3 = Semi-Smooth 4 = Rough Teeth: 1 = Absent 2 = Few 3 = Numerous Hair: 1 = Absent 2 = Present 3 = Transverse Crease Shape of Base: 1 = Depression 2 = Slight Crease Raachilla Hairs: 1 = Short 2 = Long 9. STIGMA: 1 Hairs: 1 = Few 2 = Many

/10. SEED:						2	0.0	RT	0	10	3	•	
2	Туре:	1 = Naked	2 = Covered			3656 3656	W	60 B) 3 9	., Ç	A.		
2	Hairs on Venti	ral Furrow:	1 = Absent	2 = Present									
5	Length:	3 = Mid-lor	o Mid-long (7.5 – 9.0 mm) ng (8.5 – 9.5 mm) ng to Long (9.0 – 10.5 mm)										
2	Wrinkling of H	٠.	1 = Naked 2 = Slightly W	rinkled 3:	= Semi-Wrinkled	4 = Wrinkle	d						
1	Aleurone Colo		1 = Coloriess (White or Yellov		= Blue		_						
2	Percent Aborti		·	355	MS. per 1000 Se	eds							
												·	
r	-		eptible, 2 = Resistant, 3 = Int		-								
0	Septoria	片	Net Blotch 0	Spot Blotch	[3]	Powdery Mildew							
[2]	Loose Smut	Ħ	Bacterial Blight 2	Covered Sm	ut 0	False Loose Sm	ut						
	Stem Rust	듬	_eaf Rust 0	Scab	0	Scald							
	Aster Yellows Vir	us 0 E	BSMV	BYDV		Other (Specify) _			·				
12. INSECT	: (0 = Not Tested	l, 1 = Susce	ptible, 2 = Resistant, 3 = Inte	ermediate, 4 -	Tolerant)							:	:
0	Green Bug	0 _E	English Grain Aphid 0	Chinch Bug	0	Armyworm							
0	Grasshoppers		Cerial Leaf Beetle	Other (Speci	fy)								
Hessia	n Fly Races	0 0	0 A 0 E	0	╡╴	0 c 0 G	0 0	Other S	pecify)_				_
	CAL: (0 = Not Tes	sted, 1 = Su	sceptible, 2 = Resistant, 3 =										
14. INDICA	TE WHICH VARE	ITY MOST	CLOSELY RESEMBLES TH	IAT SUBMITT	ED:								<u> </u>
	CHARACTER		NAME OF VARIE	TY	CI	HARACTER			NAME	OF V	ARIE	TÝ	<u>.</u>
Plant Tilleri	ng		Steptoe		Seed Size			More	€X				
Leaf Size	w.c		Steptoe	····	Coleoptile Elor	ngation		Mille	nnium	1	····		
Leaf Color	***		Millennium		Seedling Pigm	entation		Mille	ennium	<u> </u>			· ·
Leaf Carria	ge		Steptoe								· .	· · ·	

REFERENCES:

The following publications may be used as a reference aid for the standardization of character descriptions and terms used in this form:

- Wiebe, G.A., and D.A. Reid, 1961, Classifications of Barley Varieties Grown in the United States and Canada in 1958, Technical Bulletin No. 1224, U.S. Department of Agriculture.
- 2. Reid, D.A., and G.A. Wiebe, 1968, Barley: Origin, Botany, Culture, Winter Hardiness, Genetics, Utilization, Pests, Agriculture Handbook No. 338, U.S. Department of Agriculture, pp. 61-84.
- 3. Malting Barley Improvement Association, Milwaukee, Wisconsin, 1971, Barley Variety Dictionary.

COLOR: Nickerson's or any recognized color fan may be used to determine color of the described variety.

Exhibit D: Additional genotyping of Goldeneye barley

Materials and Methods

These molecular experiments were conducted by Dr. Shiaoman Chao at the USDA-ARS Biosciences Research Lab in Fargo (SD). In these experiments we surveyed a set of 32 barley microsatellites or SSR markers (Ramsay, et al., 2000). We will give results for eight of these SSR markers that were found to be polymorphic between Goldeneye, Aquila, Millennium and Steptoe barley genotypes. Their respective sequences are presented in Table I. We employed fluorescent-based genotyping technology using a semi-automated capillary gel system, ABI3130xl, from Applied Biosystems.

PCR reactions

The PCR reaction setup was based on the M13-tailed PCR method (Boutin-Ganache, et al, 2001) after optimization. The forward primers were modified by adding 19 bases of M13 derived sequence to their 5'end. The 19-base M13 primer was labeled with one of the four fluorescent dyes, FAM, VIC, NED and PET. For PCR reactions, 50ng of DNA template was used along with a modified forward primer, reverse primer and M13 primer labeled with one dye added at a molar ratio of 0.15:1:1. The total reaction volume was 10 microliters. The cycling condition used was based on published results for particular SSRs (Ramsay, et al., 2000).

Gel Electrophoresis and fragment analysis

The PCR products labeled with four different fluorescent dyes were pooled. The pooled samples were subjected to gel electrophoresis after adding the size standards. The gel electrophoresis was carried out on the ABI3130xl sequencer, a 16-capillary gel system. Fragment sizing and allele calling were performed using the GeneMapper v3.7 software from Applied Biosystems. The fragment size call is based on the Local Southern algorithm.

Results

We present fluorescent profiles for Goldeneye, Aquila, Millennium and Steptoe that were generated with eight sets of SSR-primers (Figures 1-8). We report polymorphism for four chromosomes of barley.

On chromosome 2 (chr 2), with the <u>Bmac0093</u> marker (Fig. 1), we found DNA fragment sizes of 174 base pairs (bp) for Goldeneye, Aquila and Millennium, and 177 bp for Steptoe.

On chr 2, with the <u>Bmag0125</u> marker (Fig. 2) DNA fragment size was the same as that in Millennium (160 bp) but differ from those of Aquila and Steptoe (154 and 152 bp, respectively).

For chr 3, with the <u>Bmag0136</u> marker (Fig. 3) the DNA fragment size observed in Goldeneye (218 bp) was shared with Millenium and Steptoe but differed from that of Aquila (220 bp).

On chr 5, we report polymorphism for four SSR markers. For <u>Bmac0303</u> (Fig.4), the allele size is 158 bp for Goldeneye, Aquila and Millennium and much smaller for Steptoe (144 bp). For <u>Bmag0337</u> (Fig. 5), the allele size for Goldeneye and Aquila (163 bp) differs from that of Millennium and Steptoe (167 bp). For <u>Bmac0096</u> (Fig. 6), Goldeneye, Aquila and Millennium have an allele size of 197 bp whereas Steptoe has an allele size of 195 bp. For <u>Bmag0223</u> marker (Fig. 7), the allele size for Goldeneye and Aquila is 176 bp as those of Millennium and Steptoe are 170 and 172 bp.

On chr 6, we found one polymorphic SSR marker, Bmgtttttt0001 (Fig. 8). The allele size is the same for Goldeneye, Aquila and Steptoe (225 bp) but it is much larger with Millennium (237bp).

Summary

We distinguish Goldeneye from Aquila with SSR markers <u>Bmag0125</u> on chr. 2 (Fig 2), and <u>Bmag0136</u> on chr 3 (Fig. 3).

Goldeneye differs from Millennium with SSR markers <u>Bmag0337</u> and <u>Bmag0223</u> on chr. 5 (Fig. 5 and 7, respectively), and marker <u>Bmgtttttt0001</u> on chr 6 (Fig. 8).

Goldeneye alleles differ from those of Steptoe for marker <u>Bmac0093</u> on chr 2 (Fig. 1), <u>Bmag0125</u> on chr 2 (Fig. 2), and for four markers of chr 5 (Bmac0303, Bamg0337, Bmac0096 and Bmag0223) (Fig. 4, 5, 6 and 7).

References

Boutin-Ganache, I., M. Raposo, M. Raymond and C.F. Deschepper (2001). M13-tailed primers improve the readability and usability of microsatellite analysis performed with two different allele-sizing methods. *BioTechniques* 31(1):25-28.

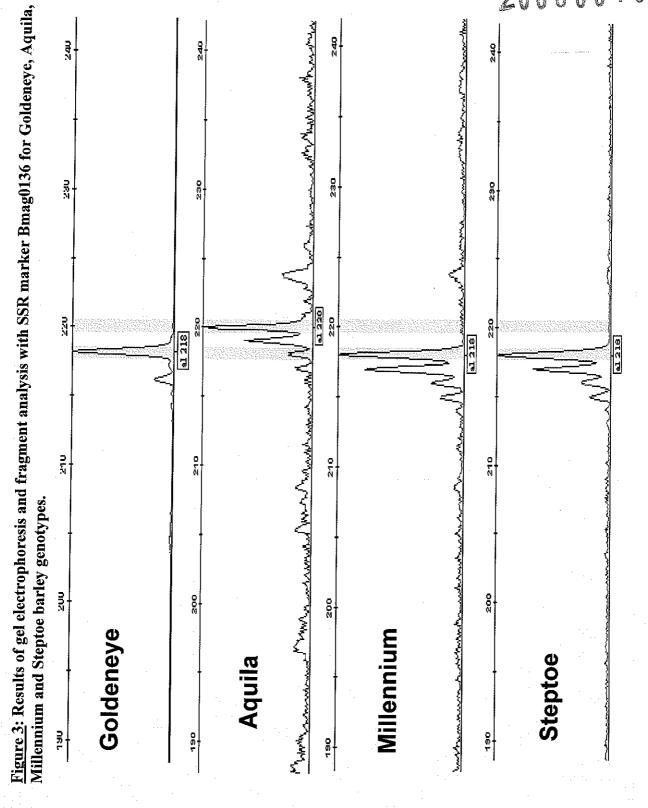
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Table 1: Microsatellite markers utilized for the genotyping of Goldeneye in a comparison to Aquila, Millennium and Steptoe barley lines. SSR abbrevations, chromosome assignment, genetic map location and sequences for forward and reverse primers are indicated.

SSR	g.	Map location (cM)	Repeat motif	Forward	Reverse
Bmac0093 Bmag0125	ий	50	(AC)24 (AG)19	CGTTTGGGACGTATCAAT AATTAGCGAGAACAAAATCAC	GGGAGTCTTGAGCCTACTG AGATAACGATGCACCACC
Bmag0136	က	20	(AG)6-(AG)10-(AG)6	GTACGCTTTCAAACCTGG	GTAGGAGGAAGAATAAGGAGG
Bmac0303	Ŋ	. 08	(AG)13(AC)21	CCTCCAAGATTAGATCTCTC	CCGTATATTAAGAAATGGTGA
Bmag0337	S	35	(AG)22	ACAAAGAGGAGTAGTACGC	GACCCATGATATGAAGATCA
Bmac0096	Ŋ	41	(AT)6(AC)16	GCTATGGCGTACTATGTATGGTTG	TCACGATGAGGTATGATCAAAGA
Bmac0223	ιΩ	69	(AG)16	TTAGTCACCCTCAACGGT	CCCCTAACTGCTGTGATG
Bmgttttt0001	9	104	(GTTTTT)5	ACACCAGAGCCTTGACTCGT	AGCAGCAACAACACAC

N. Figure 1: Results of gel electrophoresis and fragment analysis with SSR marker Bmac0093 for Aquila, Goldeneye, MA NA NA NA NA NA NA AZ AZ AZ MA NA, MA NA 130 170 5 2 N.A. Z A. 160 160 160 150 ξ. 150 Emact0093 Bmac0093 Branch093 Emac0093 Millennium and Steptoe barley genotypes. 5 8 Goldeneye Millennium Steptoe dom-1 dom-1 Aquila Gom-1 gow-1 8 8. 130 8 Esg_005_C09_fsa_3m Crag_001_A09 fsq. Al Erag_003_B09 fsa | C1 frag_007_D09 fsa_S1 120 150 120 120 200 ㅎ 180 18 200 300

200600 Figure 2: Results of gel electrophoresis and fragment analysis with SSR marker Bmag0125 for Goldeneye, Aquila, Millennium 170 160 Charged al 160 150 흡 ₹ 130 and Steptoe barley genotypes. Goldeneye Millennium Aquila Steptoe 120 120 120



Ž Figure 4: Results of gel electrophoresis and fragment analysis with SSR marker Bmac0303 for Aquila, Goldeneye, Millennium and Steptoe barley genotypes. SZ. 4 N.S. M.S. 4 4 4 160 • ž 4 4 150 4 4 **4** 5 4 Bmar0303 Emac6303 Bmac0303 Brna.c0303 G-mm-5 dom-5 Millennium Goldeneye g-wap dom-5 120 20 120 20. Steptoe Aquila frag_001_A07_fsa A5 Grag 003_B07.fsa C5 Grag_005_C07 fsa M5 Des 007_D07.fsa SS 110 200 120 100 -004 180+ 200 100 800

NA MA MA MA MA MA Figure 5: Results of gel electrophoresis and fragment analysis with SSR marker Bmag0337 for Aquila, Goldeneye, 170 • 1 789 Ź ž 9 4 160 160 160 4 4 4 150 150 Bmag0337 Bra.g0337 Britag0337 Bmag0337 Millennium and Steptoe barley genotypes. 130 130 8 130 Goldeneye dom-3 Millennium Grow-3 dom-3 g-wap Steptoe Aquila 120 120 120 8. Evag_006_C06ffsa_NE3 Erg_002_A06_fsa A3 Erag_004_B06fsa C3 frag_008_D06 fsa | S3 1000 2000-2000 3000 1000 3000 2000 1000 3000 3000 2000 1000

MA MA MA Figure 6: Results of gel electrophoresis and fragment analysis with SSR marker Bmac0096 for Aquila, Goldeneye, Z00 02. 8 ď Ž Millennium Goldeneye Steptoe Aquila Bmac6096 Brac 000 6 Bract0096 Brace 0096 Millennium and Steptoe barley genotypes. Removed at 7 Removed 117 dom-5 dent-5 gem-5 Removed dom.5 Erag_003_B07.fsa_C5 Eng_001_A07.fsa A5 Erag 005_C07.fsa | MES frag_007_D07.fsa_SS ġ

VS

180 180 8 8 Figure 7: Results of gel electrophoresis and fragment analysis with SSR marker Bmac0223 for Aquila, Goldeneye, Removed 172 at-170 Removed 72 st-170 170 ž MA MA NA NA 4 NA NA NA 4 **4** 160 160 *** 4 4 4 4 150 NA NA 4 · ₹. • Breag0223 🚵 🊵 Brag 0223 Brosg0223 Millennium and Steptoe barley genotypes. Bmag0223 dom-7 Millennium Goldeneye Aquila Steptoe don.? 110 dom.7 130 110 110 Crag_004_B08_fra _ G7 frag_002_A08 fra | A7 Ş Eag_006_C08 fsa MC 9 frag_002_A04.fsa S7 400 000 600 200 1200 99 900 200

the Mars republisher man Francisco Safer - and Albert Land Safer and Mille Sand graphy - graphy Proposite Andreas distributed by the broken the contraction of the contraction of the graphy of HANDLAND STANDAM MAN STANDARD LAND Figure 8: Results of gel electrophoresis and fragment analysis with SSR marker Bmgttttt0001 for Aquila, Goldeneye, 4 各 " Jane Bernell St. Carlotter Colored Colored State Colored ď. 230 230 • marker and hear wash Morey I want so make the conservation Hickory Hay How May Horan March March Comment Poly At 4 1 220 220 Braghillimooc. Bragdillittooo: Braghintono: Bragdillinooo Millennium and Steptoe barley genotypes. 밁 200 200 200 0 thousand the technology of a major of the forest gow-6 dom-8 dom-8 geom-8 Goldeneye Millennium · Tother acretic withingthe or confession Steptoe Aquila 130 190 190 130 Craff_004_B10 fss | C-8 Esg_002_Allofsa_A8 frag_006_C10 fra_ 1/18 8 đ. 9 604 60 9 20ģ 8 ė

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4. ADDRESS (Street and No., or R.F.D. No., City, State, and ZiP, and Country)	5. TELEPHONE (Include area code)	6. FAX (Include area code)
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b. If the original rights to variety were owned by a company(ies). YES	, is (are) the original owner(s) a U.S. bat NO If no, give name of countr	
11. Additional explanation on ownership (Trace ownership from origin	nal breeder to current owner. Use the re	everse for extra space if needed):
Goldeneye (UT95B1216-4087) was originated and preliminary dereleased by Dr. Dominique Roche, plant breedrs at the UTah Agragreement between employee and the Utah Agricultural Experime or development made by an employee are assigned to the employ the employee.	icutural Experiment Station at Utah Sta ent Station and Utah State University, a	ate University, Logan, Utah. By all rights to any invention, dicovery
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The original breeder/owner may be the individual or company who dir Act for definitions.	rected the final breeding. See Section 4	1(a)(2) of the Plant Variety Protection
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U.S. DEPARTMENT OF AGRICULTURE AGRICULTURAL MARKETING SERVICE SCIENCE AND TECHNOLOGY PLANT VARIETY PROTECTION OFFICE BELTSVILLE, MD 20705

EXHIBIT F

	DECLARATION REGARDING DEPOSIT	
NAME OF OWNER (S)	ADDRESS (Street and No. or RD No., City, State, and Zip Code and Country)	TEMPORARY OR EXPERIMENTAL DESIGNATION
Utah State University	4820 Old Main Hill	
	Logan, UT 84322	VARIETY NAME Goldeneye
NAME OF OWNER REPRESENTATIVE (S) Dr. Dominique Roche	ADDRESS (Street and No. or RD No., City, State, and Zip Code and Country)	FOR OFFICIAL USE ONLY
Dr. Dominique Roche	4820 Old Main Hill Logan, UT 84322	PVPO NUMBER 200600103

I do hereby declare that during the life of the certificate a viable sample of propagating material of the subject variety will be deposited, and replenished as needed periodically, in a public repository in the United States in accordance with the regulations established by the Plant Variety Protection Office.